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INHALATION TOXICITY STUDIES ON CIGARETTE SMOKE
II. TOBACCO SMOKE INHALATION DOSIMETRY STUDIES ON SMALL LABORATORY ANIMALS

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SUMMARY

A newly developed exposure system has been used to carry out smoke dosimetry studies on rats, mice, hamsters and guinea pigs. In all species, smoke total particulate matter (TPM) deposited in significant amounts in the lower respiratory system (LRS) at dose levels ranging from 0.515 to 1.710 mg TPM/g respiratory tissue.

Nasal deposition of smoke particulates did occur in all of the species examined.

The significance of these dosimetry data in relation to the conduct of long-term comparative inhalation toxicity studies with tobacco smoke is discussed.

INTRODUCTION

Experimental techniques for smoke inhalation studies are still being developed and several problems in this field remain to be investigated more fully. These include, for example, the choice of species for inhalation work, characterisation of smoke particulate deposition in various regions of the respiratory system of animals and smoke dosimetry studies on small species under standard conditions.

A programme was designed to investigate some dosimetry problems and to evaluate a new exposure system [1]. A large proportion of the work in-

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Abbreviations: COHb, carboxyhaemoglobin; DCBP, decachlorobiphenyl; LRS, lower respiratory system; TPM, total particulate matter.

volved exposure of rats to smoke from different cigarettes under varying conditions [2]. As part of the evaluation, it was considered worthwhile to carry out a number of experiments on rats, golden hamsters, mice and guinea pigs exposed to smoke from the same cigarette type, using the same exposure system, smoke concentration and duration of exposure throughout. It was hoped that such a comparison of species would throw some light on aspects of the more basic problems of smoke toxicity work referred to above.

MATERIALS AND METHODS

A new exposure system [1] was used for this work. During exposure animals were fitted in pairs on to the central exposure chamber in plethysmograph-type restraining tubes, the sizes of which were appropriate to the species being used. A 70 mm plain cigarette containing flue-cured tobaccos was used. Delivery of TPM from the cigarette was 41.9 mg/cigarette. During exposures smoke was generated under standard conditions (35-ml puff, 1 puff/min, to a standard butt length of 23 mm) and diluted 1 in 14 (smoke : air, v/v) before passing to the exposure chamber of the inhalation machine. Diluted smoke filled the chamber throughout the whole of the exposure period.

The technique for assessing deposition of TPM in the respiratory system of animals was based on the use of DCBP as a particulate phase marker [3]. Cigarettes were spiked with DCBP and suitably conditioned before being used. Animals, which were not preacclimatized to smoke, received a single 10-m exposure to smoke from cigarettes containing DCBP.

Following exposure, animals were rapidly killed and tissues removed for analysis of DCBP, hence TPM, retained in the various regions of the respiratory system. The respiratory system was divided into 5 regions: (*i*) skinned head (less brain), (*ii*) skinned lower jaw and tongue, (*iii*) larynx, (*iv*) trachea, and (*v*) main bronchi and lungs. Details of the analytical techniques for detection of DCBP in tissue samples have been given elsewhere [2]. COHb levels in blood samples taken immediately following exposure were determined using an IL CO-Oximeter (Instrumentation Laboratories Ltd.).

RESULTS

Monitoring of the exposure system showed that between the butt end of the cigarette and the entrance to the exposure chamber there was loss of 10.3% of TPM leaving the cigarette in mainstream smoke. During exposures the average chamber smoke concentration was 7.67 mg/l.

Amounts of TPM deposited in various regions of the respiratory system of the animals exposed under similar conditions and mean COHb levels are shown in Table I. The values for stomach samples are shown for 3 of the 4 species.

The particulate phase marker was detected in all respiratory system samples taken from all species exposed to smoke containing DCBP. For any species mean TPM deposition was always heaviest in the lungs of the ani-

TABLE I

MASS DISTRIBUTION OF TPM IN DIFFERENT SPECIES FOLLOWING 10-min EXPOSURE TO DILUTED CIGARETTE SMOKE

Figures shown for each species are mass mean deposition (S.D.), and % of total respiratory system deposition.

Species	Number of aminals	Mean body weight (g)	Head (μg) (%)	Lower jaw (μg) (%)	Larynx (μg) (%)	Trachea (μg) (%)	Lungs (μg) (%)	Total [a] respiratory system (μg)	Stomach (μg)	Blood [b] COHb (%)
Rat	6	214	83.06	64.5	47.9	34.8	596.1	826.4	—	26.5
		(49)	(42.6)	(21.0)	(46.8)	(19.5)	(254.2)	(254.3)	—	(5.1)
			10.0	7.8	5.8	4.2	72.1			n = 10
Mouse [c]	5	17	106.9	37.7	14.8	5.3	228.4	393.2	40.0	61.3
		(1)	(27.7)	(20.7)	(10.6)	(2.5)	(87.2)	(69.6)	(14.3)	(4.6)
			27.2	9.6	3.8	1.4	58.1			n = 5
Golden hamster	6	101	106.9	35.6	44.8	27.9	526.0	740.9	302.9	44.1
		(2)	(62.0)	(41.5)	(41.7)	(30.1)	(198.7)	(278.5)	(139.3)	(10.2)
			14.4	4.8	6.1	3.8	71.0			n = 8
Guinea pig	5	321	245.5	29.3	25.1	32.0	1836.5	2168.5	57.3	34.4
		(22)	(56.7)	(20.1)	(9.3)	(7.4)	(328.4)	(328.4)	(58.7)	(3.9)
			11.3	1.4	1.2	1.5	84.7			n = 7

[a] Head, lower jaw, larynx, trachea, lungs.

[b] Mean includes values for extra animals exposed to 'blank' cigarettes.

[c] 3 animals died during the exposure.

mals, showing effective penetration of smoke particulate phase into the distal parts of the respiratory system.

Calculation of a simple distribution pattern shows that TPM deposition in the upper respiratory system was to be most marked in the mouse (36.8%), least marked in the guinea pig (12.7%) and intermediate between these extremes in rats (17.8%) and hamsters (19.2%). It is interesting to note that in all species except the rat, TPM deposition was more marked in the head (predominantly nasal) than the lower jaw (predominantly oral) region. In the rat, this difference of TPM deposition in the two regions of the head was less pronounced.

It is not particularly useful to compare directly absolute TPM loads in the various tissues from the four species as shown in Table I, because of differences in bodyweight and hence distinct differences in tissue weights in various animals. It is probably more appropriate to express TPM dose in terms of a unit weight for each of the various lower respiratory tissues. This makes comparison of the dose level achieved in the different species more meaningful. Obviously, TPM dose to head regions expressed as particulate weight per unit weight of tissue would not be at all useful. Table II shows summary TPM dosimetry information for the LRS only, for the 4 species.

If the LRS is considered as a whole, TPM loading per unit weight of tissue was highest in the mouse and lowest in the rat, with the hamster and guinea pig showing a very similar intermediate dose level. Because most particulate marker was always detected in the lungs of all animals, this species ranking applies also to lung-only deposition. The calculated TPM dose to the larynx and trachea was apparently much more variable, due at least in part to the lower absolute amount of TPM marker present in these regions and the larger

TABLE II

MEAN TPM DOSE (AS μg/g TISSUE) FOR VARIOUS REGIONS OF THE LOWER RESPIRATORY SYSTEM (LRS [a]) IN RATS, MICE, GOLDEN HAMSTERS AND GUINEA PIGS

Figures shown for each species are mean and (S.D.)

Species	Larynx (μg/g)	Trachea (μg/g)	Lungs (μg/g)	LRS (μg/g)
Rat	442.9 (360.0)	310.6 (119.0)	549.4 (290.7)	515.2 (250.5)
Mouse	1873.6 (1180.5)	437.4 (269.6)	1798.9 (535.8)	1710.3 (465.4)
Golden hamster	754.8 (677.6)	859.0 (900.3)	986.1 (363.7)	970.8 (388.3)
Guinea pig	213.2 (144.5)	275.7 (99.5)	1061.4 (207.3)	946.7 (211.0)

[a] larynx + trachea + lungs.

TABLE III

FOR 4 SPECIES, THE RATIO OF HIGHEST TO LOWEST TPM DOSE (AS μg/g OF TISSUE) DETECTED IN VARIOUS REGIONS OF THE LOWER RESPIRATORY SYSTEM

Species	Larynx	Trachea	Lungs	Total LRS
Rat	12.2	5.3	6.0	3.9
Mouse	6.5	13.6	2.2	2.0
Guinea pig	10.7	3.0	1.7	1.7
Golden hamster	28.8	37.0	3.3	3.8

error inevitable in removing, cleaning and weighing these relatively small tissue samples. Generally, there was no marked indication of heavier dosing of the larynx of animals compared with other respiratory regions. Only in the mouse was the mean TPM dose/g of laryngeal tissue marginally higher than for the lung region.

A simple index of variability of dosing to the various regions is given in Table III. This index confirms that TPM dose to the larynx and trachea was very variable for all species. Variability of dose retained by the lungs was less than for larynx and trachea. If the lower respiratory system is considered as a whole, animals showed a 2- to 4-fold range between highest and lowest TPM dose detected in the complete system. The guinea pig particularly and the mouse showed least variability. In this series at least, the rat and also the hamster showed a similar approximately 4-fold variability in dose level.

Exposure of a range of species under similar conditions produced a range in blood COHb levels. Not surprisingly, direct comparison of blood COHb levels against total respiratory system TPM deposited in animals shows no clear relation between the two factors in the different species. However, it is interesting to compare blood COHb level against TPM dose in the lower respiratory system, expressed as a weight of particulate matter per unit weight of tissue, for those animals for which both values are available. Fig. 1 shows this relationship for *all* species after exposure to smoke.

For any one species the range of COHb levels and TPM dose tended to be limited. If all data are considered together, over the complete range of values the correlation between TPM dose to the LRS and blood COHb level is quite striking and is highly significant ($r = 0.795$, significant at 99.95% level). Fig. 1 is particularly interesting since it is a composite picture for different species with a range of mean bodyweights from 17 to 231 g and absolute TPM deposition levels ranging from roughly 150 μg to 2500 μg in the lower respiratory system.

Three mice died during exposure. Two of these showed the highest COHb

LRS = larynx + trachea + lungs

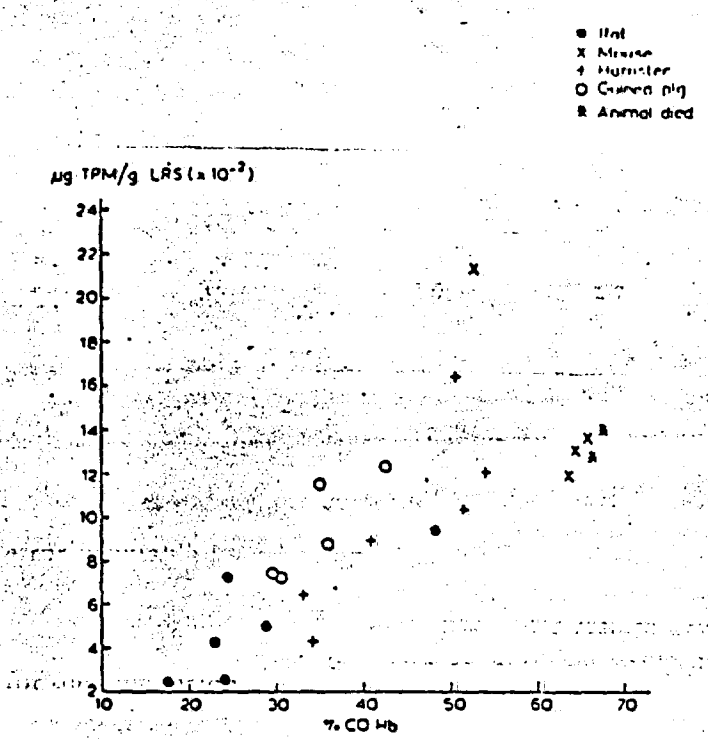

Fig. 1. The relation between TPM dose to lower respiratory system and COHb level in 4 small animal species.

levels recorded in animals, and a high TPM dose to the respiratory system. Whether one of these factors was more important than the other in limiting tolerance to exposure is not clear at present.

DISCUSSION

Information is presented in this paper on dosimetry studies carried out on the more commonly used small laboratory species. In the attempt to deal with several species, information on any particular one is necessarily limited. Yet the results lead to a number of interesting observations.

Effective penetration of smoke beyond the upper respiratory system was achieved in all species tested, with the highest mass deposition of TPM and TPM dose/g of tissue being recorded for lung samples. Deposition of TPM in the head of animals ranged from 12.7% to 36.8% of recorded TPM load in the complete respiratory system of guinea pigs and mice respectively.

The weight of evidence in this paper and in the majority of other studies on smoke particulate distribution in animals (Table IV), suggests that deposition in the head of small species does not seriously impair effective TPM dosing of the lower respiratory system of animals during smoke exposure. Lower respiratory system TPM dose levels recorded in this report were

TABLE IV

TPM DISTRIBUTION PATTERNS RECORDED FOR THE RESPIRATORY SYSTEMS OF SMALL LABORATORY ANIMALS

Species	Particulate phase marker	Number of animals	% Distribution of marker		Reference
			Head	Lower respiratory system	
Mouse	[^{14}C]hexadecane	8	70.8	29.2	[4]
	DCBP	20	33.3	66.6	[3]
	DCBP	5	36.8	63.2	This paper
Rabbit	^{74}As	6	25.7	74.2 [b]	[5]
Guinea pig	DCBP	5	12.7	87.4	This paper
European hamster	[^{14}C]dotriacontane	2	24.5	75.5	[6]
		29	23.3	77.9	[7]
Syrian golden hamster	[^{14}C]hexadecane	10	48.3	51.7	[8]
	[^{14}C]dotriacontane	2	60.4 [a]	39.6	[6]
	[^{14}C]dotriacontane	29	26.3	73.7	[7]
	DCBP	6	19.2	80.9	This paper
Rat	[^{14}C]dotriacontane	20	23.3	76.7	[9]
	DCBP	6	17.8	82.2	This paper
	DCBP	33	7.3	92.7	[2]

[a] This observation was not confirmed by a more extensive study conducted subsequently [7].
[b] Results probably suspect due to formation of arsine.

highest in the mouse, which also showed the highest percentage TPM deposition in the head.

Significant amounts of TPM were recorded in the lower jaw samples of all species. Rats, which were observed breathing through the mouth during exposure, showed heavy TPM deposition in lower jaw samples. Deposition on the jaw samples compared with the upper part of the head, which contained the nasal region, was less marked though still clear in the mouse and hamster. In the guinea pig, deposition in the head was more predominant in the upper nasal region than in the mouth. There does seem to be both physiological and anatomical evidence that the guinea pig breathes through the mouth only when very severely stressed [10]. Other species may not be obligate nose breathers during smoke exposure, as is often suggested.

Expression of TPM dose per unit weight of respiratory tissue and measurement of blood COHb levels in animals give the opportunity of assessing acute toxicity of smoke in these terms, rather than the more vague but commonly used indices based on percentage dilution of smoke and duration of exposure of animals. More precise methods of doing comparative toxicity

studies on smoke from different cigarettes, based on exact measurement of dose of smoke to animals, are also a clear possibility.

Data from dosimetry studies on rats and hamsters carried out by another laboratory show TPM dose levels to the respiratory system which compare with those found in our work. Other workers have found TPM doses to rats and hamsters of 0.27 and 0.25 mg/lung respectively, for animals exposed under similar conditions [11]. Guerin and Nettesheim showed clearly that for animals with similar bodyweights there were no major differences in mass TPM deposition in rats and hamsters, if deposition is expressed purely on the basis of TPM/lung.

It is possible to use the above figures to make some estimate if TPM dose/g of lung tissue, using relative lung weights of 0.79% and 0.496% bodyweight [12] for the rat and hamster respectively. The corresponding dose level figures for the two species from the different studies are shown in Table V.

Differences in absolute dose level are no doubt due to differences in exposure system, cigarette type, smoke dilution, exposure regime etc., in the two studies. Values calculated from ORNL data do seem to agree with our finding that for animals exposed under similar conditions, the TPM dose/g lung is greater for the hamster than for the rat. Our work indicated a dose ratio for hamsters : rats exposed under the same conditions of 1.88 : 1, compared with the estimated ratio of 2.06 : 1 from the ORNL figures. If the augmented ORNL figures are accepted as being reasonable estimates of TPM dose level, the two sets of values are remarkably close for relative dose levels obtained in different laboratories using significantly different exposure procedures.

For the combined data from all species, we showed a significant correlation between TPM dose/g of LRS and COHb level. The correlation between TPM dose and COHb level is clear for a range of small animals. Our figures indicate a linear relationship which suggests that no preferential absorption of COHb or deposition of TPM in the lung of animals occurred during exposure. For rats a similar correlation has been shown, with the same level of

TABLE V

TPM MASS DEPOSITION IN LUNGS AND TPM DEPOSITED PER GRAM OF TISSUE IN RAT AND HAMSTER: COMPARISON OF DATA FROM STUDIES CARRIED OUT IN TWO LABORATORIES

Species	Total TPM deposited/lung or (LRS) (mg)		TPM dose/unit weight lung or (LRS) tissue (mg/g tissue)	
	ORNL [a]	This report	ORNL [a]	This report
Rat	0.27	0.68	0.207	0.515
Hamster	0.25	0.60	0.427	0.971

[a] Oak Ridge National Laboratory.

significance, for lung TPM dose and total respiratory volume during exposure to smoke [11].

Because of the limited number of observations which we have in total, and for the individual species in particular, our results show that TPM dose could be predicted from blood COHb levels only within fairly wide limits. Nevertheless, the relationship between COHb and TPM shown in Fig. 1 is interesting because it covers a number of species. Further work on larger scale would be worthwhile to define clearly for one or for all species the reliability of an estimate of COHb level as an index of TPM dose to the lungs of animals. It is worth pointing out that the precise TPM/COHb relationship is likely to differ according to cigarette type or the system used for exposure of animals.

Under similar conditions of exposure, at least on the particular machine used for our work, TPM dose as μg/g LRS was least in the rat compared with hamsters, guinea pigs and mice. The rat also showed considerable variability in TPM levels recorded in the respiratory system. More extensive observations on rats [2] indicate that a 4-fold range in TPM dose achieved by animals exposed under similar conditions is common for this species.

A limited number of observations on guinea pigs indicate that relatively high TPM dose levels can be achieved in this species. The animal tolerates exposure to smoke extremely well and does not appear to be particularly irritated by smoke, as do all other species. The consistency of dose levels achieved in the guinea pig reflect the behaviour and steady breathing pattern observed for this species during exposure.

Of course, the choice of species for inhalation toxicity studies will not be determined entirely on the basis of dosimetry data. The availability of such information should, however, allow a more rational choice of animal for such experiments.

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